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IN SITU UTILIZATION OF UREA IN THE EUPHOTIC ZONE OF THE TROPICAL ATLANTIC

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Abstract: *In situ* urea uptake has been studied at three stations in the tropical Atlantic Ocean. The turnover times of urea were determined. In the mixed layer they averaged one day or less. They increased with depth.

The assimilation rates (assimilation/total uptake) were very low, many being less than 5 %, which together with direct measurements, indicates that phytoplankton was responsible for the major part of urea degradation to CO_2, with subsequent nitrogen assimilation, mainly in the nitrate depleted layer of the euphotic zone.

INTRODUCTION

Urea-decomposing organisms are known to be present in both freshwater and marine environments (ZoBell, 1946). Carpenter, Remsen & Watson (1972) and Remsen, Carpenter & Schroeder (1972) have shown that turnover times for urea, calculated from average values, were 25 days for freshwater and 4 days in brackish water ($S < 5$ ‰). Measurements of turnover times in oceanic waters are few. Remsen, Carpenter & Schroeder (1974) give values of 59 and 98 days in the north Atlantic Ocean. Few data are available for tropical oceanic areas where nitrogen is generally the limiting nutrient in the mixed layer (Dugdale & Goering, 1967). In these areas, nitrate seems to be an insignificant source of nitrogen in the mixed layer (Eppley *et al.*, 1973); ammonia and urea, the waste products of zooplankton and nekton appear to be the main source of nitrogen for phytoplankton.

The purposes of this study were to determine the assimilation rates and the turnover times for urea in the euphotic zone of three tropical Atlantic areas.

MATERIALS AND METHODS

Temperature was recorded *in situ* with an STDO sond system (Bisset Bermann) coupled with a Hewlett Packard computer. The samples of sea water were collected with 1.7 l PVC bottles on a Rosette sampler (General Oceanics); nutrient analyses were made on an autoanalyser apparatus (Technicon) as described in Strickland & Parsons (1972). For chlorophyll *a* measurements, 170 ml of sea water were filtered through Gelman type-A glass fibre filters with a very low suction pressure (75 mmHg). After grinding and extraction in 90 % acetone, the chlorophyll *a* was determined according to Yentsch & Menzel (1963) using a Turner fluorometer. Calibration was with pure chlorophyll *a* (Sigma).

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The urea-decomposing activity was determined as follows. Immediately after sampling, 140 ml sterile glass flasks were filled with sea water. A solution of labelled urea was prepared; it contained 0.92 pmole and 0.052 μCi of ^{14}C labelled urea (CEA, France) per ml. This solution was made with filtered and irradiated (UV) sea water to ensure that any trace of urea in the solution was oxidized to CO_2 and NH_3. The amount of labelled urea added was extremely low because the tracer isotope must be added in quantities negligible compared with the ambient concentration. 0.5 ml of this solution was added to each sample; incubations were made from 7 h to 17 h. After incubation, the samples were filtered through Gelman type-A glass-fibre filters to remove the urea which has been incorporated in the cells. The filters were rinsed with 5 ml of filtered sea water, dried, and stored in scintillation vials at -20 °C. 50 ml of the filtrate were acidified to pH 2.5 by 0.5 N HCl and gassed for 15 min to blow off the CO_2 produced by the hydrolysis of urea; this CO_2 contains the $^{14}CO_2$ from the labelled urea. After aeration, 5 ml aliquot portions were stored in scintillation vials at -20 °C. In the laboratory, the radioactivity was counted on a liquid scintillation spectrometer (Intertechnique SL 30); 8 ml of scintillator fluid (toluene+PPO+POPOP) were added on the filters, and 6 ml of Instagel (Packard) to the filtrates.

Controls

For initial radioactivity introduced in the samples and for urea breakdown without biological processes (samples killed with 1 ml of 20 % formalin) controls were run with each set of samples. The turnover times were calculated by difference from the residual radioactivity in the controls at the end of the incubation after acidification and aeration, and that of samples according to the following formula: $T_o = Q_i \cdot t/(R_c - R_s)\ 24$, where Q_i is the initial radioactivity in the samples and controls, R_c the residual radioactivity in the controls, R_s the residual radioactivity in the samples and t the incubation time. It is assumed that the degradation and production of urea is not light dependent, which is not strictly true (Pages, pers. comm.).

Limitation of the method: maximum measurable turnover times

Q_i averaged 59100 cpm (S.D.$_q$ = 2600) and R_c 55700 cpm (S.D.$_c$ = 1337); the difference is not significant ($P < 0.05$). The abiotic degradation is, therefore, negligible. Since R_c has a narrow dispersion the difference, $R_c - R_s$, will indicate the biological activity with good accuracy; however, to be sure that this difference is significantly different from zero, the values less than 2 S.D.$_c$ have not been retained; the incubation time being routinely 9 h, the maximum measurable turnover time was 7.8 days.

RESULTS

VERTICAL STRUCTURES OF THE AREAS STUDIED

This study was carried out in February 1975 on *R.V. Capricorne* at three stations in

the tropical Atlantic Ocean. Sation A (9° 25′ S; 9° 30′ E) was occupied 5 days; it was in the Angola Dome (Mazeika, 1967) an area where during this season the thermocline is near the surface (12–25 m) and the temperature gradient in the thermocline very sharp (> 1 °C/m) (Fig. 1); the chlorophyll *a* distribution has a typical and important

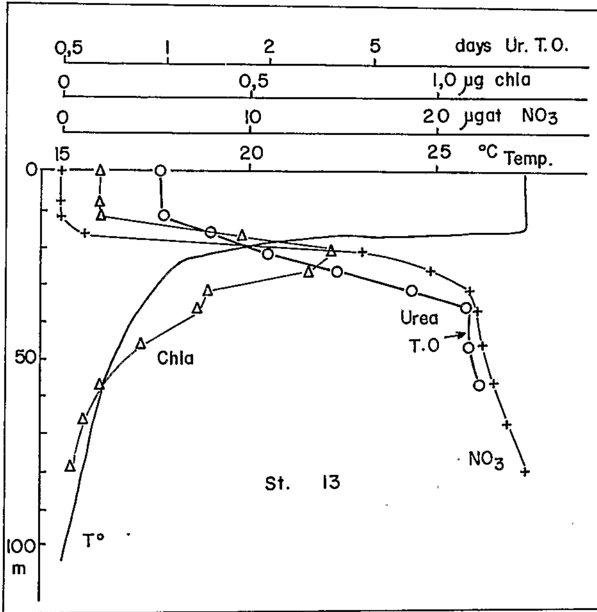

Fig. 1. Vertical distribution of temperature, nitrate, chlorophyll *a* and urea turnover time at a typical station in the Angola Dome (Station A).

maximum in the thermocline. It is a productive area because the nitrate depleted layer is thin (20–30 m) and the 'nutricline' (nutrient gradient) well illuminated. Station B (11 ° 00′ S: 5 ° 25′ W) was occupied 3 days; it was chosen because the mixed layer is deeper (40 m) than at Station A and the thermocline less sharp (0.2 °C/m) (Fig. 2). The chlorophyll *a* maximum is deeper and less pronounced than at Station A. The production is less because the nutrient depleted layer is thick (60–75 m) and the 'nutricline' weakly illuminated. Station C (0° 30′ S: 4 ° 30′ W) was occupied for 3 days and was chosen for a study of the Lomonosov Current which flows eastward and is the equivalent of the Cromwell Current for the Atlantic Ocean. At this station the thermocline is near the surface but not simple (Fig. 3). The gradient of the thermocline and the nutricline depth have values intermediate between Stations A and B.

UREA ASSIMILATION

The radioactivity on the filters may be considered as urea which has been assimilated by the cells during the incubation and not yet degraded. The assimilation rates

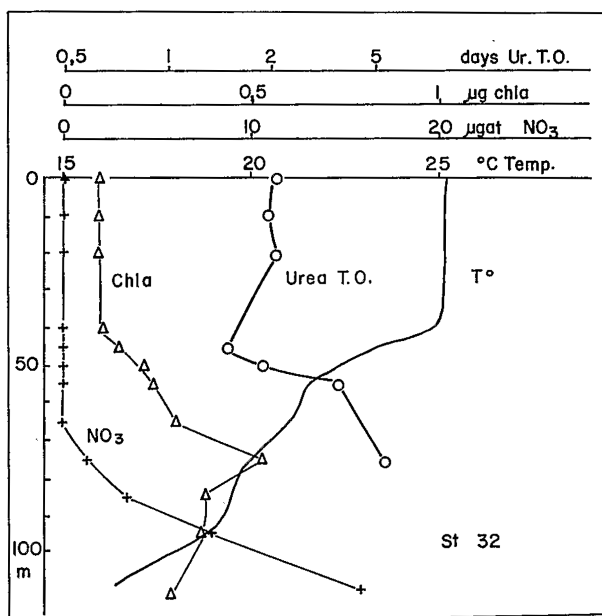

Fig. 2. Vertical distribution of temperature, nitrate, chlorophyll *a* and urea turnover time at a typical station north of St Helena Island (Station B).

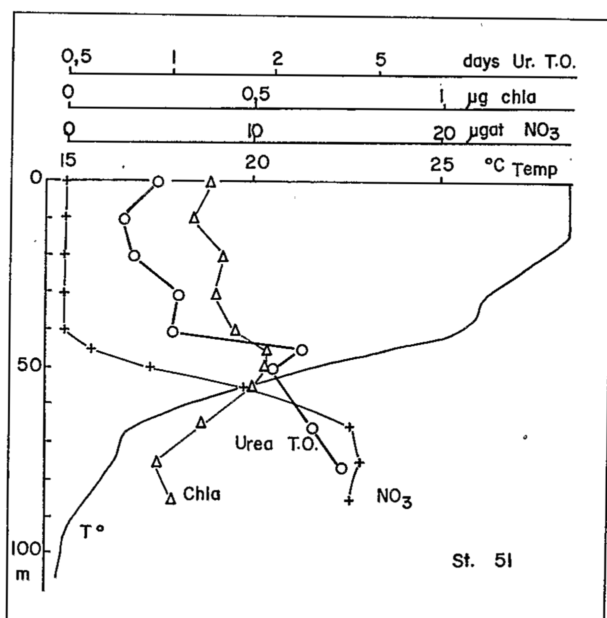

Fig. 3. Vertical distribution of temperature, nitrate, chlorophyll *a* and urea turnover time at a typical station on the Equator (Station C).

TABLE I

Turnover times and assimilation rates for urea at three stations in the tropical Atlantic Ocean: Stations 5 to 21 are the Angola Dome (Station A in the text), Stations 32 and 36 to the north of St Helena Island (Station B in the text) and Stations 40, 47 and 51 on the Equator (Station C in the text).

St. no.	Depth (m)	Turnover time J	Assim./ Tot. upt. %	St. no.	Depth (m)	Turnover time D	Assim./ Tot. upt. %
	0	1.14	5.02		0	2.10	ε
	8	0.60	1.27		10	2.00	0.73
	15	0.98	0.38		20	2.10	ε
5	25	0.53	1.67	32	40	2.70	0.72
	30	1.61	2.77		45	1.50	0.43
	40	> 8.0	5.27		50	1.90	0.78
	50	> 8.0	ε		55	3.20	ε
	70	> 8.0	ε		75	5.40	0.65
	0	–	–		0	3.60	ε
	12	0.58	ε		20	1.17	ε
	17	1.10	1.29		30	0.84	1.10
	22	2.30	8.33		40	0.88	0.20
9	32	2.30	9.60	36	50	6.30	0.83
	42	6.30	10.80		60	5.10	2.38
	52	6.80	2.80		70	2.50	1.45
	62	> 8.0	ε		80	> 8.0	ε
					90	5.10	ε
					120	6.40	ε
	0	0.99	1.49		0	1.50	0.18
	12	1.00	0.16		10	2.60	0.44
	17	1.36	1.31		20	1.10	0.41
	22	2.00	0.95		30	2.50	ε
13	27	3.20	3.18	40	40	1.70	0.19
	37	7.8	3.76		50	2.70	0.80
	47	7.9	2.84		60	2.90	0.67
	57	> 8.0	2.79		70	1.70	1.20
					80	1.50	1.03
					90	1.90	0.96
	0	0.69	0.79		0	2.00	0.95
	15	1.18	1.30		10	0.89	ε
	20	1.30	0.87		20	0.61	0.40
	25	1.50	1.56		30	0.67	0.20
17	30	2.40	2.92	47	40	2.10	0.38
	40	4.20	4.64		45	2.50	ε
	50	6.40	1.23		50	2.30	1.44
	60	> 8.0	2.64		55	5.50	ε
					65	2.70	ε
	0	0.56	1.77		0	0.92	ε
	15	1.00	0.85		10	0.73	0.21
	25	4.40	5.13		20	0.78	1.71
	30	2.00	2.40		30	1.10	1.13
	40	2.70	3.23		40	1.02	1.43
21	50	3.30	1.57	51	45	> 8.0	12.70
	60	3.80	7.33		50	> 8.0	1.74
					55	> 8.0	ε
					65	2.60	ε
					75	3.20	0.86

(assimilation/total uptake) are low (Table I). They rarely exceed 10 % and are generally less than 5 %; no correlation of assimilation rates with depth or any other parameter is evident.

UREA DEGRADATION: TURNOVER TIMES

Station A. In the mixed layer the turnover times are very short; urea is depleted in 24 h or less. The turnover times increase sharply with depth (Fig. 4); values greater than 8 days have been plotted arbitrarily at 10 days.

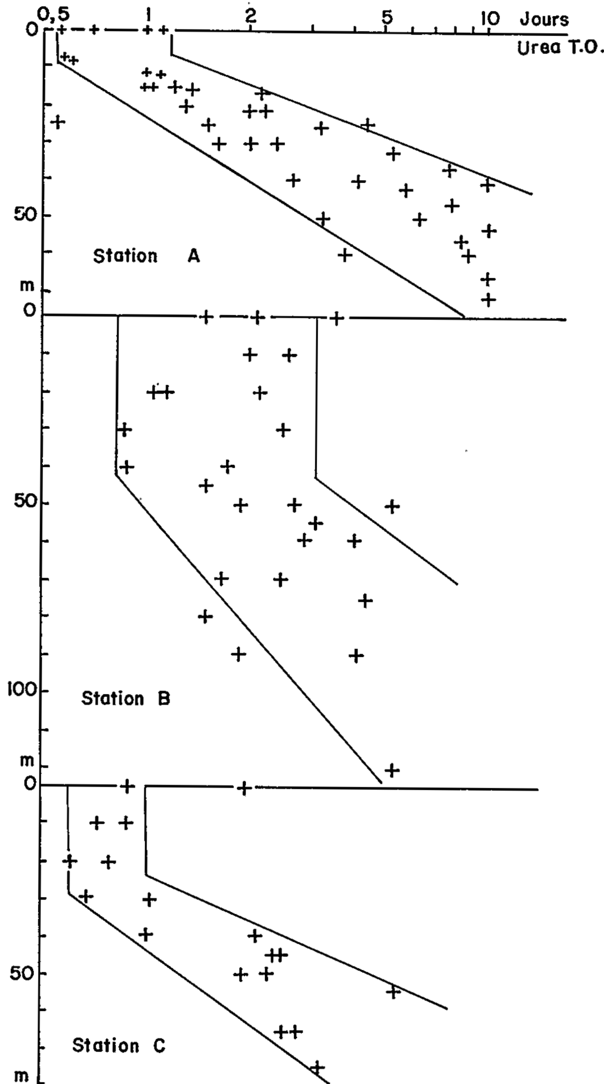

Fig. 4. Vertical distribution of urea turnover times at three stations in the tropical Atlantic Ocean.

Station B. The turnover times are minimum in the mixed layer, but with higher values and more variation than in the Dome area (Fig. 4). The average value in the mixed layer is 2 days; they increase with depth from the top of the thermocline and the slope is less than at Station A.

Station C. The turnover times are again minimum in the mixed layer, with low values less than 24 h. There is again an increase with depth in the thermocline (Fig. 4).

WHAT ARE THE ORGANISMS RESPONSIBLE FOR UREA UTILIZATION?

In the thermocline at Station C we have measured simultaneously the urea uptake, the glucose uptake (^{14}C method), and primary production ($^{14}CO_2$ uptake) with successive filtrations on 8 μm and 0.3 μm pore size filters. All these measurements were made on the same sample. We have assumed that primary production is only due to phytoplankton and glucose uptake mainly to heterotrophic bacterial activity.

Although the urea assimilation is not well represented in the total urea uptake (5 % or less), we have compared the 3 parallel incubations (Table II). In primary production experiments the organisms which pass through an 8 μm filter have an average activity of 4.1 % of the total CO_2 assimilation; in glucose uptake experiments this fraction is more important (20 %). The results for urea assimilation are close to those in primary production experiments (6.3 %). Consequently at this station, the phytoplankton seems to be responsible for the major part of urea utilization.

TABLE II

Successive filtrations on 8 μm and 0.3 μm pore size filters to show the organisms responsible for urea uptake in the thermocline.

Depth (m)	Urea assimilation			Glucose assimilation			Primary production		
	0.3 μm (cp 4 min)	8 μm (cp 4 min)	< 8 μm (%)	0.3 μm (cp 4 min)	8 μm (cp 4 min)	< 8 μm (%)	0.3 μm (μg C/l/h)	8 μm (μg C/l/h)	< 8 μm (%)
20	0	2030	0.0	953	2380	28.6	0.03	0.77	3.75
30	70	1415	4.7	1289	3589	26.4	0.00	0.35	0.00
40	661	11650	5.4	417	4313	8.8	0.06	0.41	12.70
45	113	641	15.0	681	3568	16.0	0.00	0.40	0.00

DISCUSSION

The assimilation rates are very low; urea is immediately broken down to CO_2, and re-utilization by photosynthetic processes is not important. The labelled carbon found in the cells is more probably in a transient form than well incorporated in the cellular structures.

The turnover times measured in the euphotic layer are very short compared with those previously found (59 and 98 days) by Remsen, Carpenter & Schroder (1974) in the oceanic waters of the north Atlantic Ocean. Their method of estimation was not

the same as mine; they calculated the turnover times from urea decomposition rates and *in situ* concentrations. The urea decomposition rates were obtained by incubation with labelled urea at a concentration of 1.0 μmole/l. In oceanic waters such an added amount cannot be neglected: in their study the ambient urea concentrations were between 0.06 and 0.77 μmole/l. From theoretical considerations Wright (1974) has pointed out that the measured turnover time increases sharply when the tracer is of the same order of magnitude or greater than the natural concentration. Williams & Askew (1968) have found experimentally the same result with glucose. When the glucose concentration was increased from 0.54 to 5.4 μg/l, the measured turnover time increased 4-fold.

In our experiments, the labelled urea concentration was 3.3 pmole/l; the urea added behaves as a tracer but such a low concentration does not allow the measurement of turnover times longer than 8 days. If the concentration added was increased 5-fold, the urea would again behave as a tracer and the greater accuracy would allow turnover times measurements as long as 30–40 days.

The turnover times increase with depth from the top of the thermocline. For the three stations there is a logarithmic relation between *in situ* temperature and turnover time. The average Q_{10} is 7.0 (Fig. 5). Such a high Q_{10} shows that temperature is not

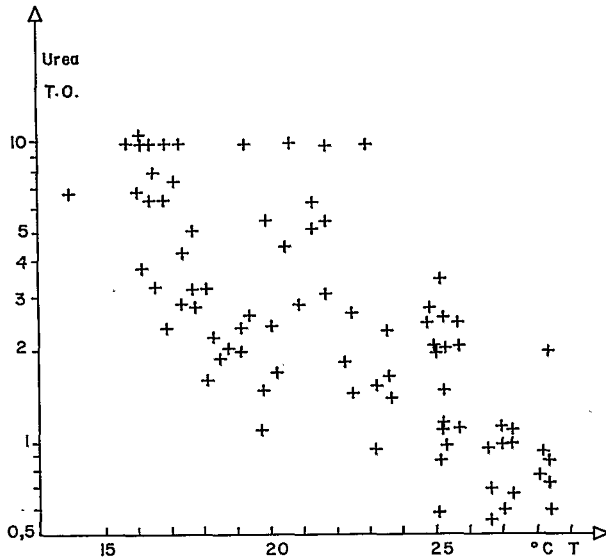

Fig. 5. Relation between urea turnover times and *in situ* temperatures; turnover times greater than 8 days have been plotted arbitrarily at 10 days.

the main factor which regulates the urea turnover. This relation does not express the direct temperature action on urea turnover but rather the importance of the thermocline in vertical distribution of the biological parameters dealing with urea cycle.

Although urea degradation to CO_2 does not reflect the nitrogen assimilation the urea turnover times are very short in the depleted nitrate layer. McCarthy (1972) found such low turnover times for urea nitrogen when nitrogen nutrients were low (< 1.0 μg-at N/l). Since turnover times give an indication of the efficiency of the biological activity, our results suggest that the mixed layer is a very dynamic system in spite of its low biomass.

Phytoplankton seems to be responsible for the major part of the urea decomposition. Carpenter, Remsen & Watson (1972) have found the same result in an estuary and in adjacent coastal waters in Georgia. Urea is an organic compound of low molecular weight; its hydrolysis reaction is weakly exothermic and does not allow ATP synthesis from ADP and phosphate so that urea is then less attractive for *sensu stricto* heterotrophic organisms than more complex organic compounds. On the other hand, it is a nitrogen component easily hydrolysable for autotrophic organisms which are under nitrogen limiting conditions.

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